The Objection under 37 CFR §1.821(b and c) Should be Withdrawn

The Examiner objected to the absence of the sequence of IκBα in the application since specific residues, i.e. 32 and 36, of this sequence are mentioned in the instant pending claims.

Applicant respectfully traverses this objection. The sequence of IκBα was well-known in the art at the time of the priority date of the present application. The IκBα sequence was published in 1991 (Haskill et al. Cell 65:1281-1289, reference C2 of IDS resubmitted herewith), available in GenBank (M69043) by April 27, 1993 (see Exhibit A), and though named "MAD-3, an I kappa B-like protein," was understood as "I kappa B alpha" at least by 1993 (see Brown et al. abstract, Exhibit A). The priority date of the present application, March 19, 1996 was five years later than the date of the Haskill et al. publication. One skilled in the art at the time of the priority date, therefore, would have had access to the IκBα sequence, and would have understood Applicant's reference to its "serine residues 32 and 36" without inclusion of its sequence in the application. A brief survey of some publications of that time reveals that many artisans who used and/or mutated the IκBα sequence had access to the sequence (e.g. through the Haskill et al. publication or through GenBank) and saw no need to actually include the complete IκBα sequence in their publications.

In addition, as the IκBα sequence referred to in the claims cites only two residues, §1.821 is not applicable to the present situation. For these reasons, this objection should be withdrawn.

Review of Information Disclosure Statement Submitted on August 12, 2002

The Examiner explained that some references listed on the IDS filed on August 12, 2002 were not considered because copies were not submitted or the references were not listed on the proper form. Applicant notes that on October 16, 2000, in the parent application, 09/406,293, Applicant filed an IDS listing all the references included in the first 5 pages of the present form 1449. The present Examiner considered those references on February 27, 2001 (see Exhibit B) and included notification of that action in an Office Action mailed on February 28, 2001. Nevertheless, for convenience, Applicant is re-submitting an IDS together with copies of references not yet considered by the Examiner for the present case.

Rejection of Claims 1-4 Under the Judicially Created Doctrine of Obviousness-type Double Patenting

Claims 1-4 are rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-7 of U.S. Patent No. 6,107,073.

Applicant will consider filing a terminal disclaimer if claims deemed to be conflicting with claims 1-7 of U.S. Patent No. 6,107,073 are indicated as being allowable.

The Rejections of the Claims under 35 U.S.C. §112, First Paragraph, Should Be Withdrawn

Claims 1-6 were rejected under 35 USC §112, first paragraph. The Examiner states that while the specification is enabling for a kinase that is activated or dependent upon ubiquitin or MEKK-1, it does not reasonably provide enablement for claims of the scope of the instant claims. The Examiner alleges that it is not taught that the kinase is active without one of these two sources of activation. The Examiner cites a paragraph on page 6 of the specification to support that conclusion.

Applicant respectfully traverses this rejection. Contrary to the Examiner's assertion that the claimed kinase is not shown to be active without activation by ubiquitination or phosphorylation by MEKK1, Applicant notes where several alternatives to these activation methods are enabled in the present application. Applicant points out that one alternative, that additional routes to activation are possible, e.g. phosphorylation by a kinase other than MEKK1, can be found on page 6 in the same paragraph cited by the Examiner. In the following paragraphs, Applicant discusses some of the alternatives to ubiquitin and MEKK1 activation presented in the specification.

An example of where Applicant enabled the kinase without ubiquitin or MEKK1 can be found on page 26, lines 1-2 of the specification. Here, Applicant describes the results of an experiment performed to understand the contribution of various ubiquitination components from a mono Q column of a HeLa cell extract (fraction I is flow-through, fraction II is 0.5 M KCl eluate) in the activation of the claimed kinase. When fraction I, containing an essential ubiquitination component, was not added to the assay mixture, the kinase activity in fraction II was reduced, but not eliminated (Fig 3A, lane 4 vs 5). Therefore, kinase activity is present without ubiquitination or MEKK1. Applicant further notes, on page 36, line 6, that concentrated extracts from uninduced HeLa cells have some activity, therefore, the application is enabling for isolating and assaying for a kinase without ubiquitination or MEKK1.

This phenomenon subsequently has been shown by others. The kinase of the present invention, now known in the art as the "IKK complex" due to its core components I kappa B kinases α, β, and γ (or NEMO) is frequently isolated as an immunoprecipitate using an anti-IKK α or γ antibody and then assayed for activity without ubiquitin or MEKK1. An immunoprecipitated IKK complex from unstimulated CD4+ T cells phosphorylated IκBα at a low level even without CD3/CD28 treatment, which greatly stimulated the activity (Fig 1 lane 1 of Khoshnan et al (2000) J. Immunol. 165:6933-6940, cited as reference F2 in IDS filed herewith). From unstimulated HeLa cells, an IKK complex with the same chromatographic elution profile as the complex from TNFα-stimulated HeLa cells and the complex of the present invention demonstrated a low level of IκBα phosphorylation without ubiquitin or MEKK1 (Fig. 1 of DiDonato et al (1997) Nature 388:548-554, cited as reference F3 in IDS filed herewith). Other scientists have reconstituted an IKK complex in yeast cells using recombinant IKK α, β, and γ. Yeast do

not display NF-κB activity nor do they express a kinase with the ability to phosphorylate IκBα at serines 32 and 36. A reconstituted complex isolated from yeast cells with a stoichiometry of $\alpha_1\beta_1\gamma_2$ had the same chromatographic elution pattern as the complex of the present invention and was active in an assay without ubiquitin or MEKK1 (Fig 1B and Fig. 3A-C of Miller and Zandi (2001) J. Biol. Chem. 276:36320-36326, cited as reference F4 in IDS filed herewith). In the figures in the Miller and Zandi article, the scientists also confirm the low level of activity of the endogenous IKK complex isolated from unstimulated HeLa cells.

In another example, on page 32 line 20-24 of the specification, Applicant discusses the use of okadaic acid, a phosphatase inhibitor, enabled on page 90, line 22 in the early stages of purification. Applicant teaches that the kinase activity is constitutive and that okadaic acid inhibits a phosphatase, which is removed from the later stages of kinase purification. In this example, at least early in the purification of the kinase, Applicant has enabled the use of a phosphatase inhibitor to assay for the kinase activity, rather than ubiquitin or MEKK1.

This possibility has been extended by others (Fig. 4 of Fu et al. (2003) J. Biol. Chem. 278:1487-1493, cited as reference F5 in IDS filed herewith). In the Fu et al. article, an IKK complex was isolated from cells which expressed an inhibitor (Tax) of serine/threonine protein phosphatase 2A. The activity of this complex was assayed without the addition of ubiquitin or MEKK1 and found to be active. Addition of the phosphatase to the assay inactivated the IKK complex, but addition of Tax or okadaic acid together with the phosphatase prevented this inactivation.

In yet another example, Applicant describes methods to initiate a stress response before cell lysis. On page 32, lines 28-30 and page 33, lines 6-12 of the specification, Applicant describes adding H_2O_2 to produce reactive oxygen intermediates (ROI) before lysis. Applicant states that the ROIs could activate the claimed kinase or inactivate a phosphatase. Later research by others attributed this phenomenon to stimulation of tyrosine kinase activity, which at least in some cells, can activate protein kinase D to activate the IKK complex (Fig. 6 of Storz and Tokier (2003) EMBO J. 22:109-120, cited as reference F6 in IDS filed herewith). In this research, the IKK complex, isolated from cells stimulated with mediators of oxidative stress (e.g. H_2O_2 or pervanadate) or with TNFα for comparison, was assayed without the addition of ubiquitin or MEKK1.

Applicant describes another method to elicit a stress response and activate the kinase. On page 37, lines 16-18 of the specification, Applicant describes experiments using extracts from HeLa cells subjected to hypotonic lysis. The IκBα kinase isolated from extracts of these cells stressed by hypotonic conditions was active without the addition of ubiquitin or MEKK1. One skilled in the art can elicit a cellular stress response and isolate an active kinase without addition of ubiquitin or MEKK1.

On page 35 line 12 through page 36 line 24 and Example 9, page100 line 26 through 101, line 17 of the specification, Applicant teaches activation of the kinase by TNF-α. In these experiments, the HeLa

cells were treated with TNF-α before lysis to isolate the claimed kinase. After rapid lysis of the cells, Applicant measured the phosphorylation of IκBα or the IκBα kinase activity of fractions of the cell extract. The kinase was activated by TNF-α without the addition of ubiquitin or MEKK1 (Figures 10A and B).

In regard to phosphorylation by MEKK1, Applicant teaches, e.g. on page 44, line 28 through page 45 line 3, that MEKK1 is a member of a family of enzymes with a conserved catalytic domain with overlapping substrates. This overlap of substrates indicates that another referenced MEKK1 enzyme also could activate the claimed kinase. One skilled in the art would be able to use one of the referenced MEKK family members in the manner described for MEKK1 in the specification and also activate the claimed kinase. Indeed, this concept has been extended by Applicant and others to yield activation by MEKK2 and MEKK3 (Yang et al. (2000) Nature Immunol. 2:620-624, cited as reference F7 in IDS filed herewith; Zhao and Lee (1999) J. Biol. Chem. 274:8355-8358, cited as reference F8 in IDS filed herewith).

While on page 6, lines 16-17 of the specification, Applicant introduced the possibility of activation of the kinase complex by kinases other than MEKK1, Applicant ruled out some possible kinases on page 42, line 25 through page 43, line 2, Example 14, page 104, lines 15-21 and Fig. 15A. However, further studies by Applicant and others have continued this work and confirmed the activation of the complex by other kinases. For example, an IKK complex chromatographically purified from unstimulated HeLa cells and assayed without ubiquitin or MEKK1 was activated by NAK (NF-κB-activating kinase, also named TANK-binding kinase 1; Fig 2f of Tojima et al. (2000) Nature 404:778-782, cited as reference F9 in IDS filed herewith). Since filing the present application, Applicant has reported that an activated TAK1 (TGF-beta activated kinase) can phosphorylate the IKK complex instead of MEKK1 (Figs 2 and 4e of Wang et al. (2001) Nature 412:346-351, cited as reference F10 in IDS filed herewith). Therefore, direct assays show activation of the IKK complex by kinases other than MEKK1, and the assays can be performed without ubiquitin.

In view of these extensive teachings, Applicant enables the scope of the instant claims pursuant to 35 USC §112, first paragraph. Applicant has enabled the isolation and characterization of an enzyme complex which phosphorylates IκBα at serines 32 and 36 and has enabled several methods to assay for and activate the complex. Since then, scientists have used the methods enabled in the present specification in combination with the level of skill in the art, have published experiments which use the kinase complex and have confirmed and/or extended the possibilities for activation enabled by the Applicant. For these reasons, Applicant requests reconsideration and withdrawal of this rejection.

CONCLUSIONS

In view of these remarks, Applicants respectfully submit that the objections and rejection of the claims under 35 U.S.C. § 112 are herein overcome and that this application is now in condition for allowance. Early notice to this effect is solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned.

This paper is being filed timely as a petition for a one-month extension of time is being filed herewith. No additional extensions of time are required. In the event any additional extensions of time are necessary, the undersigned hereby authorizes the requisite fees to be charged to Deposit Account No. 501668.

Entry of the remarks made herein is respectfully requested.

Respectfully submitted,

7 July 2003

MILLENNIUM PHARMACEUTICALS, INC.

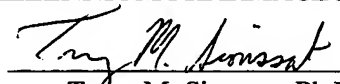
By 

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Exhibit A to Accompany the Office Action Mailed on March 7, 2003

On the following pages are the GenBank record of Accession No. M69043 (2 pages), the GenBank Sequence Revision History for M69043 (1 page), and an abstract of an article by Brown, et al. (1993) Proceedings of the National Academy of Sciences USA, 90:2532-2536 (1 page).